Mycobiology 34(2): 99-103 (2006)

Variation in Sensitivity Among Some Isolates of *Macrophomina phaseolina* Isolated from Cotton Roots to Flutolanil Fungicide

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(*Received March 2, 2006*)

Toxicity of the fungicide Flutolanil was *in vitro* tested against 20 isolates of *Macrophomina phaseolina* and cotton seedlings of ten commercial cotton cultivars. The isolates were recovered from roots of cotton plants obtained from different cotton-growing areas in Egypt. Most of the tested isolates were sensitive to Flutolanil; however, they varied in sensitivity. Twenty-five percent of the isolates were highly sensitive where IC_{50} ranged from < 1 to 5.1 $\mu g/ml$, 20% of the isolates were sensitive where IC_{50} ranged from 15 to 30 $\mu g/ml$, 45% of the isolates were moderately sensitive where IC_{50} ranged from 46 to 58.5 $\mu g/ml$, and 10% of the isolates were not much sensitive (tolerant) where IC_{50} was > 100 $\mu g/ml$. Flutolanil was very safe on both shoots and roots of the tested cultivars (IC_{50} > 100 $\mu g/ml$). Treating cotton seeds with Flutolanil resulted in highly significant ($P < 0.01$) reductions in pathogenicity of 18 isolates and a significant reduction ($P < 0.05$) in pathogenicity of isolate M_{29}. M_1 was the only isolate, which was insensitive to the application of Flutolanil. *In vivo* toxicity to Flutolanil was not correlated with its *in vitro* toxicity. However, a highly significant correlation ($r = 0.60$, $P < 0.01$) was observed between pathogenicity of isolates and the *in vivo* toxicity of the fungicide.

KEYWORDS: Cotton, Fungicide, *Macrophomina phaseolina*, Root rot

Macrophomina phaseolina (Tassi) Goid., the causal agent of charcoal rot of cotton, is of widespread distribution in the Egyptian soil, and it is easily and frequently isolated form cotton roots particularly during the late period of the growing seasons (Aly *et al.*, 2000).

M. phaseolina is a non-specialized fungus, attacking more than 500 host species (Sinclair, 1982); therefore, rotation of cotton with other crops is a questionable practice for controlling this pathogen.

Resistance to *M. phaseolina* has not been found in cotton (Hollida and Punithalingam, 1970). Sources of resistance were considered completely lacking in Pakistan (Akhtar, 1977). Aly *et al.* (1998) and Omar (2005) reported variation in susceptibility of Egyptian cottons to *M. phaseolina*; however, none of the tested cultivars showed satisfactory level of resistance.

Thus, fungicides have become indispensable for controlling *M. phaseolina* on cotton. For instance, Dwivedi and Ghaube (1985) studied effect of fungicides on the emergence and infection of cotton seedlings by *M. phaseolina* in pot experiments. They found that emergence was maximized after treatment with a TMTD soil drench and the least seedling infection occurred in soils treated with benlate, TMTD, and PCNB (Quintozene).

Chauhan (1986a) reported that control of seedling disease of cotton due to *M. phaseolina* was best and germination maximized when seeds were treated with carbendazim, followed in effectiveness by quintozene.

Chauhan (1986b) evaluated seed treatments of 7 fungicides and selected pair-wise combinations. The percentage of root rot of cotton at harvest ranged from 14 to 22% in treated plots compared with 28% in the control. Benzimidazole-derived fungicides differed in their effects on isolates of *M. phaseolina*. Benlate and carbendazim were most inhibitory but a sesame isolate was less sensitive to all fungicides.

Chauhan (1988) obtained good control of *M. phaseolina* on cotton by seed treatment with carbendazim followed by quintozene, while TMTD and catafol were less effective. A pre-sowing soil drench with quintozene and carbendazim gave very effective control.

Aly *et al.* (2001) evaluated the efficiency of monceren, Pencycuron, carboxin 200, Tolclofos-methyl, and maximum AP for controlling *M. phaseolina* on cotton, under greenhouse conditions. Monceren, Pencycuron, and Tolclofos-methyl were equally effective in increasing the percentage of surviving seedlings and the plant height; however, monceren was the only fungicide, which significantly increased dry weight of seedlings. Omar (2005) found that monceren and Tolclofos-methyl were the best performing fungicides in controlling *M. phaseolina* on cotton, under greenhouse conditions. This superiority was attributed to the following reasons: First, they were effective in increasing the percentage of surviving seedlings by 203.09 and 170.10%, respectively, regardless of the tested isolate. Second, they were also effective in in-

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creasing the height of surviving seedlings by 93.21 and 62.57%, respectively. Third, monceren and Tolclofos-methyl were effective in increasing dry weight of the surviving seedlings in 50 and 40% of the tested isolates, respectively.

The objective of this investigation was to evaluate variation in sensitivity to the fungicide Flutolanil among isolates of *M. phaseolina* isolated from cotton roots.

Materials and Methods

***In vitro* study.** A laboratory study was carried out to evaluate sensitivity of *M. phaseolina* isolates to five concentrations of the fungicide flutolanil (Flutolanil, 25% WP). Stock solution of flutolanil (500 $\mu g/ml$) was prepared by dissolving an appropriate amount in sterile distilled water. Then the tested concentrations of 1, 15, 25, 50 and 100 $\mu g/ml$ were obtained by adding the appropriate amounts of stock suspension of the fungicide to autoclaved PDA medium cooled to about 45°C. Fungicide was not added to control treatments. Fungicide-amended and non amended PDA for each isolate was poured into 9 cm diameter plates. There were five replicates (plates) for each treatment. After solidification of PDA, each plate was centrally inoculated with 5 mm-diameter disc taken from margin of actively growing fungal colony on PDA. Plates were incubated at 30 ± 3°C, and colony diameters were measured when the fungal growth completely covered plates of the control treatments. Percentage toxicity was calculated according to the following formula (Topps and Wain, 1957): percentage of inhibition of mycelial growth (I% toxicity) = [(A–B/A] × 100 where A = mean diameter of growth in the control treatment and B = mean diameter of growth of a given isolates grown on the pesticide amended PDA. Linear regression equations were fitted to logarithmic probability data of fungicide concentration and percentage of toxicity of Flutolanil-treated isolates. Slope values and IC_{50} values were interplotted (Finney, 1970). IC_{50} is the dose required to cause fungi toxic inhibition of the tested isolates by 50% in comparison with the control treatment.

Phytotoxicity test. The test was carried out according to El-Nawawy *et al.* (1972). Cotton (*Gossypium barbadence* L.) seeds of 10 commercially grown cultivars (Table 2) were dipped in water for 3 to 4 hrs. then incubate in wet cotton cloth for 24 hrs. The selected germinating seeds with their rootless were immersed slightly in the surface of agar in a test tube (1.5 × 20 cm) containing IC_{50} of Flutolanil. Percentage inhibition of both roots and shoots were measured according to the following formula: I% = [(A – B)/A] × 100 where A = length of shoot or root in the control and B = length of shoot or root in a given treatment.

***In vivo* toxicity under greenhouse conditions.** Substrate for growth of each isolate of *M. phaseolina* was prepared in 500-*ml* glass bottles; each bottle contained 50 g of sorghum grains and 40 *ml* of tap water. Contents of each bottle were autoclaved for 30 minutes. Isolate inoculum, taken from one-week-old culture on PDA, was aseptically introduced into the bottle and allowed to colonized sorghum for three weeks. The sorghum-fungus mixture of each isolate was used to infest autoclaved clay loam soil at a rate of 50 g/kg soil, and infested soil was dispensed in 10-cm-diameter clay pots. Flutolanil was added to slightly moist seeds (cultivar Giza 89) at a rate of 2 g/kg seeds. The seeds were shaken thoroughly in plastic bag for five minutes and allowed to dry before being planted in the infested pots of each isolate. Untreated seeds were planted in the infested pots of each isolate as a control. There were three replicates (pots) for each treatment. The pots were randomly distributed on a greenhouse bench under temperature regime ranged form 22 ± 3°C to 42.5 ± 3.5°C. Percentages of dead seedlings were recorded 45 days after planting.

A randomized complete block design with three replicates was used in the greenhouse study. Percentage data were transformed into arc sine angles before carrying out analysis of variance (ANOVA) to produce approximately constant variance. Least significant difference (LSD) was applied for comparing between untreated and treated seeds of each isolate. ANOVA and correlation analysis were performed with a computerized program.

Results and Discussion

List of fungal strains of *M. phaseolina* and its geographic origin have shown in Table 1. It is quite clear that the isolates under the study nearly obtained from all over the country (Egypt). Most of the tested isolates were sensitive to Flutolanil; however, they varied in sensitivity (Table 2). In general, it was possible to classify these isolates into four distinct categories: highly sensitive, sensitive, moderately sensitive, and poorly sensitive (tolerant). The highly sensitive category included M_4, M_5, M_{13}, M_{18}, and M_{25} (25% of the isolates) where IC_{50} ranged from < 1 to 5.1 $\mu g/ml$. The sensitive category included M_{15}, M_{19}, M_{20} and M_{29} (20% of the isolates where IC_{50} ranged from 15 to 30 $\mu g/ml$.

The moderately sensitive category included M_1, M_2, M_3, M_8, M_{10}, M_{11}, M_{14}, M_{17} and M_{28} (45% of the isolates) where IC_{50} ranged from 46 to 58.5 $\mu g/ml$. The poorly sensitive (tolerant) category included M_7 and M_{12} (10% of the isolates) where IC_{50} was > 100 $\mu g/ml$. This high level of variability in sensitivity to Flutolanil among *M. phaseolina* isolates is in conformity with the results of Omar (2005) who fount *in vivo* interaction between isolates of *M. phaseolina* and seed-dressing fungicides-that is, the

Table 1. List of fungal isolates of *Macrophomina phaseolina* used in this study

Isolate number	Geographic origin
M_1	Minufiya-Sirs El-Laian
M_2	Assiut El-Kosiya
M_3	Giza-Giza
M_4	Gharbiya-Tanta
M_5	Minufiya-Shoubra Blol
M_7	Gharbiya-Tanta
M_8	Beheira-Damanhour
M_{10}	Damietta-Kafr Saad
M_{11}	Daqahliya-Minyet El-Nasr
M_{12}	Minufiya-Berkit El-Saba
M_{13}	Kafr El-Sheikh El-Riyad
M_{14}	Assiut-Manfalout
M_{15}	Giza-Giza
M_{17}	Sharqiya-Zagazig
M_{18}	Daqahliya-Aga
M_{19}	Sharqiya-Diyarb Nigm
M_{20}	Beheira-Kom Hamada
M_{25}	El-Minya-Bani Mazar
M_{28}	Sohag-Tahta
M_{29}	Sohag-Tema

Table 2. IC_{50} values and slopes of Flutolanil fungicide, dosage - linear growth response curves for *Macrophomina phaseolina* isolates in fungicide amended with PDA after 72 hrs of growth at 28°C

Isolate no.	IC_{50} ($\mu g/ml$)*	Slope
M_1	58.5	0.511
M_2	53	0.512
M_3	52	0.320
M_4	1	0.045
M_5	5.1	0.229
M_7	>100	0.305
M_8	51	0.500
M_{10}	50.5	0.440
M_{11}	50	0.864
M_{12}	>100	0.460
M_{13}	<1	0.193
M_{14}	46	0.290
M_{15}	15	0.376
M_{17}	50	0.299
M_{18}	<1	0.120
M_{19}	18	0.179
M_{20}	30	0.374
M_{25}	<1	0.144
M_{28}	48	0.360
M_{29}	17	0.241

*IC_{50} ($\mu g/ml$) and slopes were determined by extrapolation from log - dosage probit.

isolates responded differently to the application of fungicides.

Treating cotton seeds with Flutolanil resulted in highly significant ($P < 0.01$) reductions in pathogenicity of 18 isolates and a significant reduction ($P < 0.05$) in pathogenicity of M_{29}. *In vivo* toxicity of flutolanil against *M. phaseolina* isolates ranged from 31.24 to 90%. M_1 was the only isolate, which was insensitive to the application of Flutolanil (Table 3). Data of Table 4 showed that Flutolanil was very safe on both shoots and roots of the tested cultivars (IC_{50} > 100 $\mu g/ml$). These results are almost in agreement with the results of (Ismail and Aly 1997; Zein, *et al*., 1999; Ismail, 2004).

Although *In vivo* toxicity of flutolanil against *M. phaseolina* isolates ranged from 31.24 to 90%; however, *in vivo* toxicity of Flutolanil was not correlated with its *in vitro* toxicity (Table 5). This lack of correlation may be attributed to the fact that *in vitro* efficiency of a fungicide is an outcome of a direct interaction between the chemical composition of the fungicide and the genotype of the pathogen. Under greenhouse conditions, other factors may interfere modifying the outcome of the *in vitro* interac-

Table 3. Effect of the seed-dressing Flutolanil on pathogenicity of 20 isolates of *M. phaseolina* on cotton seedlings (cultivar Giza 89) under greenhouse condition

Isolate no.	Percentage of dead seedlings (Pathogenicity (%)[a])		Reduction in pathogenicity[b]	*In vivo* toxicity[c]
	Untreated seed (T_1)	Flutolanil-treated seed (T_2)		
M_1	53.33	36.67	16.66 N.S	31.24
M_2	76.67	23.33	53.34**	69.58
M_3	63.33	20.00	43.33**	68.42
M_4	93.33	43.33	50.00**	53.57
M_5	96.67	40.00	56.67**	58.62
M_7	93.33	33.33	60.00*	64.29
M_8	80.00	20.00	60.00**	75.00
M_{10}	53.33	30.00	23.33**	43.75
M_{11}	66.67	13.33	53.34**	80.01
M_{12}	86.67	30.00	56.67**	65.39
M_{13}	100.00	16.67	83.33**	83.33
M_{14}	100.00	36.67	63.33**	63.33
M_{15}	86.67	23.33	63.37**	73.08
M_{17}	86.67	16.67	70.00**	80.77
M_{18}	80.00	30.00	50.00**	62.50
M_{19}	100.00	23.33	76.67**	76.67
M_{20}	100.00	10.00	90.00**	90.00
M_{25}	66.67	26.67	40.00**	60.00
M_{28}	80.00	13.33	66.67**	83.34
M_{29}	56.67	36.67	20.00*	35.29

[a]Pathogenicity is the percentage of dead seedlings after 45 days from planting date. Percentage data were transformed into arc sine angles before carrying ANOVA to produce approximately constant variance.
[b]Reduction in pathogenicity was non significant (N.S), significant at $P<0.05$(*), or highly significant $P<0.01$ (**) according to LSD test. LSD was 17.11 ($P<0.05$) or 22.98 ($P<0.01$).
[c]Toxicity was calculated according to the following formula: $[(T_1-T_2/T_1)\times100$, where T1 is the number of untreated seeds and T2 is the number of Flutolanil-treated seeds.

Table 4. IC_{50} and slopes values of the fungicide Flutolanil on root and shoot systems of cotton seedling on different cultivars

Cultivar	Root system		Shoot system	
	IC_{50} ($\mu g/ml$)[a]	Slope	IC_{50} ($\mu g/ml$)	Slope
Giza86	>100	0.77	>100	0.73
Giza88	>100	0.70	>100	0.66
Giza80	>100	0.68	>100	0.79
Giza70	>100	0.65	>100	0.72
Giza90	>100	0.80	>100	0.53
Giza89	>100	0.75	>100	0.59
Giza83	>100	0.63	>100	0.68
Giza45	>100	0.87	>100	0.69
Giza91	>100	0.55	>100	0.60
Giza85	>100	0.79	>100	0.52

[a]The concentration of Flutolanil (dose) that is required to cause phytotoxic inhibition of the cultivar by 50% in comparison with control treatment.

Table 5. Correlation coefficients among pathogenicity of *M. phaseolina* isolates, *in vitro* toxicity; and *in vivo* toxicity of Flutolanil against these isolates

Variable	1	2	3
1. Pathogenicity[a]	–	−0.145	0.596**[d]
2. *In vitro* toxicity[b]	−0.145	–	0.006
3. *In vivo* toxicity[c]	0.596**[d]	0.006	–

[a]Percentage of dead seedlings when untreated seeds were planted in *M. phaseolina*-infested soil.
[b]*In vitro* toxicity was expressed as IC_{50} ($\mu g/ml$) under pure culture conditions.
[c]*In vivo* toxicity is the magnitude of reduction in pathogenicity under greenhouse condition when Flutolanil - treated seeds were planted in the infested soil.
[d]Correlation coefficient is significant at $p<0.01$ (**).

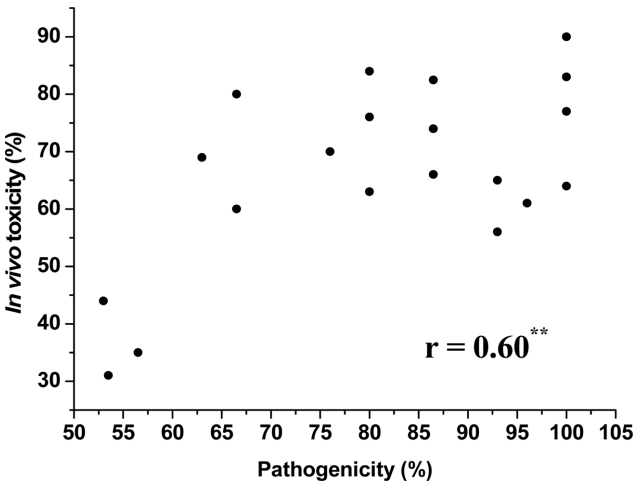

Fig. 1. Correlation coefficient between isolates pathogenicity and the *in vivo* toxicity of the fungicide.

tion. These factors may include (stability of the fungicide in soil (Snel *et al.*, 1970; Buchenauer, 1975; Huppatz *et al.*, 1984), activity of fungicide against ineffective propagules of the isolates (Weinhold and Bowman, 1974; Ktaria and Grover, 1975; Huppatz *et al.*, 1983), the host cultivar (Katoria and Verma, 1990), and the inoculum density (Gaber *et al.*, 1979). This result indicates that *in vitro* performance of Flutolanil is of no practical value in predicting its *in vivo* performance under greenhouse conditions.

A highly significant correlation was observed between pathogenicity of isolates and the *in vivo* toxicity of the fungicide (Table 5 and Fig. 1). This correlation indicates that Flutolanil had the highest inhibitory against the most pathogenic isolates of *M. phaseolina*, while it showed the lowest inhibitory effect against the least pathogenic isolates.

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